Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 12 (2): 604 - 619, June 2026 e-ISSN: 2685-7332

Potential of *Bacillus* sp. (BTH-22) in Several Alternative Media for Controlling Fusarium Stalk Rot in Maize

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Submitted March 23 Th 2026, and Accepted June 07 Th 2026

Abstract

Background: *Fusarium stalk rot caused by Fusarium spp. remains a major threat to maize production. Although Bacillus spp. are effective biological control agents, their widespread application is hindered by the high cost of synthetic propagation media. However, whether inexpensive plant-based media can simultaneously support efficient bacterial propagation and preserve biocontrol efficacy against Fusarium stalk rot remains unclear. This study aimed to evaluate the potential of Bacillus sp. BTH-22 propagated in several alternative liquid media for controlling Fusarium stalk rot in maize.* **Methodology:** *The study was conducted through both in vitro and in vivo experiments in screen house using rice washing water, mung bean broth, and cowpea broth as alternative propagation media. Parameters observed included bacterial population density, antagonistic activity against Fusarium sp., disease incubation period, and disease intensity.* **Findings:** *The results demonstrated that all alternative media supported the growth of Bacillus sp. BTH-22, with cowpea broth producing the highest bacterial population density (2.85×10^{10} CFU mL^{-1}). In vitro antagonism assays revealed that Bacillus sp. BTH-22 effectively inhibited the growth of Fusarium sp., with the highest inhibition observed in cowpea broth medium (0.521%). In vivo application significantly delayed symptom development and reduced disease intensity in maize plants. The lowest disease intensity was recorded in the mung bean broth treatment (0.12%), compared with the control treatment (0.75%).* **Contribution:** *These findings indicate that alternative liquid media, particularly cowpea and mung bean broth, can serve as cost-effective and sustainable media for propagating Bacillus sp. BTH-22 as a biological control agent against fusarium stalk rot in maize.*

Keywords: *Alternative Media; Bacillus sp.; Biological Control; Fusarium sp.; Maize*

https://doi.org/10.36987/jpbn.v12i2.9342

INTRODUCTION

One of the challenges in corn cultivation is stem rot caused by the fungus Fusarium sp (Wang et al., 2025). Stem rot in corn plants can reduce corn yield by up to 50% (Han et al., 2015), and in severe cases, it can lead to plant death (Octaviani et al., 2023). Fusarium is a significant disease in corn because it can be transmitted through seed or soil (Solórzano et al., 2024). This pathogen causes rot in the stem, cob, and kernels. Infection begins with symptoms on the leaves, which turn pale, the plant then wilts and the stem becomes soft (Zhang et al., 2022).

Plant disesase control to manage fusarium stem rot have primarily involved the use of chemical fungicides (Wang et al., 2020). Fungicides are widely used by farmers because they are considered effective and practical for managis fusarium stem rot. However, continuous use of chemical fungicides can have negative impacts on the environment and surrounding ecosystems. One control method that can be used to manage fusarium stem rot is the use of biological agents (Lu et al., 2023). Some commonly used biological agents include Bacillus sp.

The propagation of *Bacillus* sp. is currently often carried out using synthetic agar media and synthetic liquid media. The use of synthetic agar and liquid media is costly when used for propagation. Therefore, natural materials with the potential to serve as mass propagation media for *Bacillus* sp. are needed-materials that are easily accessible and affordable. The use of natural media can also serve as an alternative in the utilization of natural resources. Microorganisms require metal elements for their growth such as sodium, potassium, calcium, magnesium, manganese, iron, zinc, copper, phosphorus, cobalt, hydrogen, oxygen, and sulfur (Thohari et al., 2019). Some types of alternatives growth media that can be used include organic liquid waste, such as cowpea beans broth, mung beans broth, and rice washing water.

Alternative media used for bacterial propagation must be nutritionally adequate. Based on research conducted by Salsabila et al., (2023), alternative culture media made from cowpea flour can be used for the growth of *Escherichia coli* because it contains 26.42% protein, 63.04% carbohydrate, 1.44% fat, 4.96% moisture, 6.46% crude fiber, and 4.13% ash, all of which support bacterial growth. Patricia et al., (2022) stated that 100 grams of mung beans contain 20 milligrams of calcium, 0.1 grams of thiamine, 2.4 milligrams of vitamin C, 62.2 grams of carbohydrates, 22 grams of protein, and 1.20 grams of fat. Based on research conducted by Ningsih et al., (2024), a liquid medium derived from mung bean extract can be used for the propagation of *Pseudomonas fluorescens*, yielding a total colony count of 6.08 × 10^8 CFU/mL. Meanwhile, rice washing water can be used as a growth medium for *Acetobacter xylinum* with optimal growth because it contains thiamine, amino acids, lysine, and other nutrients that can stimulate bacterial growth (Nion et al., 2016)

Mung bean broth , cowpea broth, and rice washing water have considerable potential as alternative media for the propagation of *Bacillus* sp. due to their relatively high protein content (Naik & Kerkar, 2024; Fadhilah et al., 2022; Santos et al., 2021; Annan et al., 2010). Therefore, these three materials should be utilized optimally to reduce the cost of *Bacillus* sp. propagation and may also be applied as a control agent for *Fusarium* disease in corn plants. Furthermore, the use of these materials supports environmentally friendly and sustainable agricultural practices.

Collectively, these studies demonstrate that plant-derived substrates and agricultural by-products can provide sufficient nutrients to support the growth of various beneficial bacteria. However, the suitability of each substrate depends on its nutritional composition and the physiological requirements of the target microorganism, resulting in considerable variation in bacterial growth performance among different species. Moreover, previous studies have primarily focused on bacterial propagation, whereas evidence regarding whether these alternative media can simultaneously support high-quality propagation of *Bacillus* sp. while preserving its biocontrol efficacy against *Fusarium* stem rot in maize remains scarce. This knowledge gap highlights the need to evaluate not only bacterial population density but also the biological performance of propagated cells under practical disease management conditions.

METHOD

Research Location and Material

This study was held from June to October 2025 at the Plant Health Laboratory, Faculty of Agriculture, "Veteran" National Development University of East Java, and *in vivo* in the screenhouse of "Veteran" National Development University of East Java. The equipment required for this study includes petri dishes, test tubes, inoculation loops, beakers, Erlenmeyer flasks, measuring cylinders, an analytical balance, sieves, a stove, a Bunsen burner, a Laminar Air Flow cabinet, a glass stirrer, a spatula, and a measuring tape. The materials required for this study include the *Bacillus* sp. BTH-22, *Fusarium* sp. collection isolates, Potato Dextrose Agar (PDA) medium, Nutrient Agar (NA) medium, Nutrient Broth (NB) medium, distilled water, rice, mung beans, cowpeas, aluminum foil, plastic wrap, 5% formalin, polybags, corn seeds, soil, and compost fertilizer.

The *in vitro* study used a Complete Randomized Factorial Design with two factors: differences in culture media and differences in the age of the *Bacillus* sp. culture. The design consisted of 4 treatments with 1 experiment, repeated 3 times therefore, 30 trial units were obtained. The *in vivo* study used a Completely Randomized Design (CRD) with one factor: differences in the type of *Bacillus* sp. culture medium. This design consisted of 5 treatments with 5 replicates, therefore, 125 trial units were obtained.

Procedure

The research stages began with isolate rejuvenation, preparation of alternative media, bacterial inoculation into the alternative media, population density calculation, *in vitro* antagonism testing, *in vivo* antagonism testing, and observation of disease severity.

Isolate Revitalization

The *Bacillus* sp. BTH-22 collection by Arika Purnawati and *Fusarium* sp. collection by Adibul Akrom bacterial isolates Akrom et al., (2024) to be used in the study were first rejuvenated on slanted NA medium for bacteria and PDA for fungi.

Preparation of Alternative Media

The preparation of the alternative growth medium using rice washing water was carried out by weighing 250 g of unpolished rice and washing it with 150 ml of distilled water (Yunus et al., 2017), then measuring the pH of the solution using a pH meter. The washing water was filtered and transferred to an Erlenmeyer flask, then sterilized using an autoclave at 121 °C for 20 minutes at 1.5 atm pressure.

Preparation of the alternative growth medium using mung bean cooking water was performed by weighing 20 g of green beans, then washing them thoroughly 2–3 times. The washed mung beans were placed in a pot, 100 ml of distilled water was added, and the mixture was boiled until it reached a rolling boil. Once boiling, add a sugar solution by dissolving 25 g of sugar in 50 ml of distilled water, then stir until mixed and bring to a boil (Apriliyah & Ilmiah, 2024). After boiling, let the mung bean broth cool to room temperature, then measure the pH of the solution using a pH meter. Next, filter the mung bean broth and transfer it to a beaker. It is then sterilized using an autoclave at 121 °C for 20 minutes.

The preparation of the alternative growth medium using cowpea bean broth begins by sorting the cowpea beans to separate the good ones from the damaged ones; 20 g of cowpea are used, which are then washed with water for 2–3 rinses (Fallo et al., 2021). The cowpea peas are placed in a pot and 100 ml of distilled water is added. The black-eyed peas are boiled until they come to a boil, then 25 g of sugar is added and dissolved in 50 ml of distilled water. After boiling, the pH of the solution is measured using a pH meter; the mixture is then filtered and transferred to an Erlenmeyer flask, sealed, and sterilized in an autoclave at 121 °C for 20 minutes.

Inoculation Bacillus sp, Bth-22 to each alternative media

Inoculation of *Bacillus* sp. Bth-22 into each alternative medium was performed by pouring 10 mL of *Nutrient Broth* containing *Bacillus* sp. Bth-22. After inoculation, each medium was covered, incubated for 3 × 24 hours, and agitated using *a shaker* at 100 rpm (Saputra et al., 2023).

In Vitro Antagonism Assay

The antagonism test was performed using the double culture method to assess the inhibitory activity of *Bacillus* sp. in each alternative medium at intervals of 24 hours, 48 hours, and 72 hours. Each 100 ml of the prepared liquid medium was solidified by adding 2 g of agar. The medium was then sterilized in an autoclave at 121 °C for 20 minutes. Then, one loop of *Bacillus* sp. from the culture of each liquid medium was taken and a dilution series was performed up to 10^8. *Fusarium* sp. was taken in a 1-loop amount, followed by a dilution series up to 10^6 (Halwiyah et al., 2019). Next, filter paper that had been looped was soaked in the suspension of each bacterium and fungus, then inoculated onto PDA medium at a distance of 3 cm from the edge and 3 cm from the *Fusarium* sp. pathogen. After incubation for 7 days, the inhibition zones formed between *Bacillus* sp. and *Fusarium* sp. were observed.

In Vivo Test

The plant medium used consisted of compost and soil in a 1:1 ratio. Before mixing the soil and compost, the soil was treated with 5% formalin at a rate of 2.5 ml/kg of soil (Musafa et al., 2015), then covered and incubated for 7 days. After 7 days, the sterilized growing medium was opened and air-dried for 7 days to remove residual formalin. The soil was then mixed with compost and placed into 30 x 30 cm polybags. The seeds used in this study were Syngenta variety NK 212; prior to use, the seeds were soaked in 70% alcohol for 1 minute.

Application of the *Bacillus* sp. suspension was performed 1 day before planting at a concentration of 10^8 CFU/mL, with 10 mL per polybag. The prepared seeds were planted using the hole-planting method. Pathogen inoculation was conducted 7 days after planting. Pathogen inoculation was performed using a 7-day-old pure culture at a density 10^6 spores/mL. Pathogen inoculation on the plants was carried out by pouring 10 ml of *Fusarium* sp. suspension per polybag in total 125 polybag onto the surface of the growing medium.

Observation of Colony Count

Colony count observation was performed using the *Total Plate Count* method, which involved taking 1 ml from each liquid alternative medium. A dilution series was then conducted at a dilution factor 10^{-6}. From the results of this dilution series, 0.1 ml of the *Bacillus* sp. suspension was taken and inoculated into solid NA medium. The plates were then incubated for 24 hours, and the number of colonies was calculated using the formula by Nufus et al., (2016) as follows:

$$\text{Number of colonies } (CFU/mL) = \sum number\ of\ colonies\ in\ petridish \times \frac{1}{dilution\ factor}$$

Inhibition Zone

The observation of the inhibitory activity of *Bacillus* sp. was conducted by measuring the diameter of the colonies formed from the antagonistic test. The formula refers to Safitri et al., (2019):

$$\text{DH } (\%) = \frac{R1 - R2}{R1} \times 100\%$$

Description,
DH: Inhibitory Zone (%)
R1: Radius of the pathogenic fungal colony moving away from the antagonistic bacterial colony.
R2: Radius of the pathogenic fungal colony approaching the antagonistic bacterial colony.

Incubation period

Observation of the incubation period was conducted by recording the onset of fusarium stem rot symptoms in corn plants, characterized by wilting of the leaves and the lower stem turning brown. Observation were made daily until the plants reached 35 days after sowing.

Disease Intensity

Disease Intensity observation were calculated from the onset of symptoms (7days after inoculation) at 7 day intervals up to 35 DAP using the following formula refers to Mugiastuti et al., (2019). The diseases percentage score of plant dagame caused by fusarium wilt disease based on (Akrom et al., 2024).

$$IP\ (\%) = \frac{\sum(n_i \times v_i)}{Z \times N} \times 100\%$$

Description,
IP: Disease intensity (%)
N_i: Number of leaves in each attack category i
V_i: Scale value for each attack category i
Z: Scale value of the highest attack category
N: Number of plants observed

Table 1. Disease Intensity refers to Akrom et al., (2024)

Scale	Disease intensity (%)
0	Healty plants, no symptoms
1	0-25% of leaves wilted
2	26-50% of leaves wilted
3	51-75% of leaves wilted
4	76-100% of leaves wilted

Data Analysis

The data obtained from the study were analyzed using analysis of variance (ANOVA) in IBM SPSS Statistics 25 to determine the effects of each treatment. When significant differences were detected, the analysis was followed by the Honestly Significant Difference (HSD) test at the 5% significance level. Data from the in vitro antagonism test were analyzed using IBM SPSS Statistics 25 and RStudio software.

RESULT AND DISCUSSION

Colony Counts on Various Alternative Media

The total plate count results showed that the highest colony count was observed in 48 hours in the boiled cowpea bean water medium, reaching 2.85×10^{10} CFU/mL, followed by the boiled mung bean water medium at 2.58×10^{10} CFU/mL and the rice washing water medium at 2.32×10^{10} CFU/mL. Despite the differences in colony counts, all three alternative media were able to support the growth of *Bacillus* sp. Bth-22. These findings are consistent with those reported by Thakur et al., (2009), who stated that nutrient composition and environmental conditions influence the optimal growth of microorganisms.

The growth medium for *Bacillus* sp. must provide essential nutrients required for microbial growth, particularly carbohydrates and proteins, which are utilized for cellular synthesis and metabolism. Bacterial growth is also influenced by the pH of the medium. According to Graumann (2007), *Bacillus* sp. exhibits optimal growth at a pH range of 7–8. Based on the protein content analysis, boiled cowpea wa (M3) contained

0.47% protein, boiled mung bean water contained 0.22% protein, and rice washing water contained 0.14% protein.

Table 2. Colony Count of *Bacillus* sp. BTH-22

Media	Colony Count (CFU/mL)		
	24 Hours	**48 Hours**	**72 Hours**
Rice washing water	$4{,}0 \times 10^{9}$	$2{,}32 \times 10^{10}$	$8{,}3 \times 10^{9}$
Mung bean broth	$4{,}6 \times 10^{9}$	$2{,}58 \times 10^{10}$	$9{,}9 \times 10^{9}$
Cowpea broth	$5{,}8 \times 10^{9}$	$2{,}85 \times 10^{10}$	$1{,}37 \times 10^{10}$

The higher colony counts observed in the boiled cowpea water and boiled mung bean water media may be attributed to their higher protein content compared with rice washing water. Proteins are subsequently metabolized by bacteria into nitrogen sources, amino acids, and peptides. Amino acids entering the cell can be directly utilized for the synthesis of new proteins, whereas peptides undergo further metabolic processes broken down by intracellular peptidases into free amino acids (Madigan et al., 2018). In addition to direct uptake, *Bacillus* also possesses the ability to produce extracellular protease enzymes. These enzymes hydrolyze complex proteins present in peptone into smaller molecules (Nelson & Cox, 2017).

This mechanism increases nitrogen availability in the medium and enables *Bacillus* sp. to maximize the utilization of medium components as nutrient and energy sources. The utilization of these proteins allows *Bacillus* sp. to synthesize cellular components, replicate DNA, and ultimately proliferate through binary fission. Therefore, protein availability in the growth medium plays a crucial role in the growth and reproductive efficiency of *Bacillus* bacteria.

Based on the observational results, all alternative media can be used as propagation media because they meet the standards established by the Ministry of Agriculture Regulation (2011) concerning organic fertilizers, biofertilizers, and soil amendments. According to this regulation, the quality standard for free-living or endophytic bacteria in liquid form as bioagents is $\geq 10^{8}$ CFU/mL. Nutrient availability is a key of factor in bacterial growth.

In Vitro Antagonism Test

These macroscopic observations indicate that antagonistic bacteria play an important role in inhibiting the growth of pathogenic fungal mycelia through nutrient competition and antibiosis mechanisms. Differences in colony size indicate the effectiveness of antagonistic bacteria in suppressing the growth of pathogenic fungi during the incubation period, suggesting their potential as biological control agents against *Fusarium* sp. Hersanti et al., (2021) stated that the width of the inhibition zone is influenced by environmental conditions, culture medium composition, temperature, incubation time, and bacterial age.

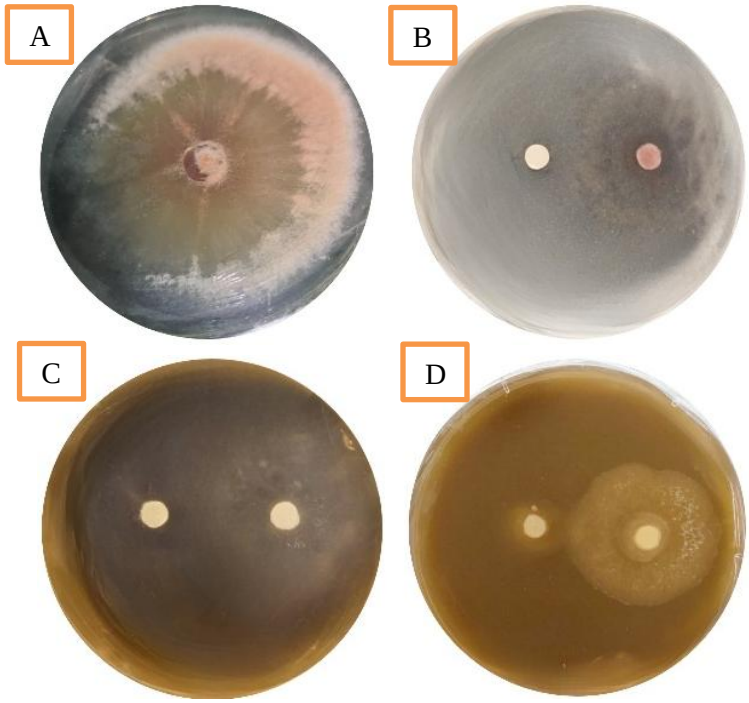

Figure 1. In Vitro Antagonism Result on Several Alternative Media
a) Control (only *Fusarium* sp.), b) Rice washing water medium, c) Mung bean broth medium, d) Cowpea broth medium.

Table 3. Antagonism Test of *Bacillus* sp. BTH-22

Treatment	Observation of Inhibitory Activity (%) Day						
	1	**2**	**3**	**4**	**5**	**6**	**7**
M0	0	0 b	0 b	0 c	0 c	0 c	0 c
M1	0.169	0.206 a	0.26 a	0.316 b	0.383 b	0.428 b	0.474 b
M2	0.183	0.23 a	0.283 a	0.338 ab	0.399 ab	0.438 b	0.485 ab
M3	0.224	0.271 a	0.332 a	0.386 a	0.434 a	0.485 a	0.521 a
HSD 5%	ns	0.229	0.166	0.111	0.089	0.074	0.063
J0	0	0	0	0	0	0	0
J1	0.231	0.277	0.325	0.368	0.42	0.463	0.503
J2	0.178	0.201	0.283	0.338	0.4	0.45	0.495
J3	0.167	0.227	0.268	0.334	0.396	0.439	0.483
HSD 5%	ns	ns	ns	ns	ns	ns	ns

Notes : Values followed by the same letter in the same column indicate no significant difference based on the HSD 5% test. M0: Control (Only *Fusarium* sp.), M1: Rice washing water medium, M2: Mung bean boiling water medium, M3: Cowpea boiling water medium. J0: Control (Only *Fusarium* sp.), J1: 24-hour culture age, J2: 48-hour culture age, J3: 72-hour culture age.

Based on the results of the ANOVA and the 5% Honest Significant Difference (HSD) post-hoc test presented in Table 3, a significant difference was observed among the treatment groups with respect to the propagation media beginning at 2 days after inoculation (DAI). The highest inhibition percentage was recorded in treatment M3

(0.52%), followed by treatment M2 (0.48%) and treatment M1 (0.47%). In contrast, the culture age factor (J0–J3) did not show a significant effect on the inhibitory activity of the antagonistic bacteria. Overall, the results indicate that no significant interaction occurred between the treatments, suggesting that each factor acted independently. The effectiveness of the M treatment in inhibiting pathogens was not influenced by the J treatment level, and vice versa. In other words, the increase in inhibitory activity was primarily determined by the M factor alone, without synergistic or antagonistic interactions between the two factors.

Physiologically, these findings suggest that standard media possess a more optimal nutritional composition—particularly in terms of the carbon-to-nitrogen (C/N) ratio—which is more readily available for bacterial cellular metabolism compared with organic waste-based media. Although alternative media tend to contain relatively high levels of carbon and nitrogen, their nutrient composition is generally less stable and less optimal for secondary metabolite production. Consequently, while microbial growth may still occur, the production of secondary metabolite compounds remains relatively low Chen et al., (2023). According to Zulfarina et al., (2022), each isolate shows variation in the formation of clear zones due to differences in the types of secondary metabolites produced. This is because the size of the inhibition zone depends on the ability of each endophytic bacterial isolate to produce antibacterial compounds.

Statistically, all treatments—rice washing water (M1), mung bean decoction (M2), and cowpea decoction (M3)—were classified within the same significance group (a) and did not differ significantly from one another. However, medium M3 (cowpea decoction) tended to produce slightly higher inhibitory activity values than M1 and M2 at the end of the observation period. This phenomenon may be associated with the protein content and specific nitrogen precursors present in legumes, which play important roles in the biosynthesis of secondary metabolites and hydrolytic enzymes by *Bacillus* sp. BTH-22. In contrast, the lower inhibitory activity observed in the rice washing water medium (M1) was likely due to the predominance of soluble carbohydrates, which favor vegetative cell growth rather than the production of antimicrobial compounds.

Evaluated based on culture age, variations in incubation time between 24 h (J1), 48 h (J2), and 72 h (J3) did not significantly affect the inhibitory activity across the three alternative media tested. This finding suggests that *Bacillus* sp. BTH-22 likely reached the stationary phase or the peak production of antagonistic metabolites within 24 h of incubation. Nutrient limitations in the alternative media were presumed to be a limiting factor, preventing the production of inhibitory compounds from increasing linearly despite prolonged incubation periods. Nevertheless, the continuous increase in inhibitory activity observed until the seventh day across all treatments indicates that antagonistic mechanisms, including space competition and antibiosis, remained active, although at relatively low intensity. Overall, no significant differences were observed among the treatments.

Based on microscopic observations of the hyphae in the *Fusarium* sp. antagonism test, abnormal growth was observed, such as curled and bent hyphae. These changes in hyphal structure indicate the presence of antibiosis activity by *Bacillus* sp. Bth-22. *Bacillus* sp. bacteria inhibit fungal growth by secreting the enzyme chitinase,

and *Bacillus* sp. can also produce secondary metabolites that inhibit the growth of *Fusarium* sp. This is consistent with the research by Indriani et al., (2023), which states that *Bacillus* sp. has the ability to produce various antimicrobial compounds that effectively inhibit the growth of pathogenic fungi. The chitinase enzyme produced by *Bacillus* sp. plays a role in the degradation of chitin, the main component of fungal cell walls. Additionally, the secondary metabolites produced by *Bacillus* sp. can disrupt the permeability of fungal cell membranes, inhibit the activity of essential enzymes, and inhibit protein synthesis, thereby disrupting fungal cell metabolism.

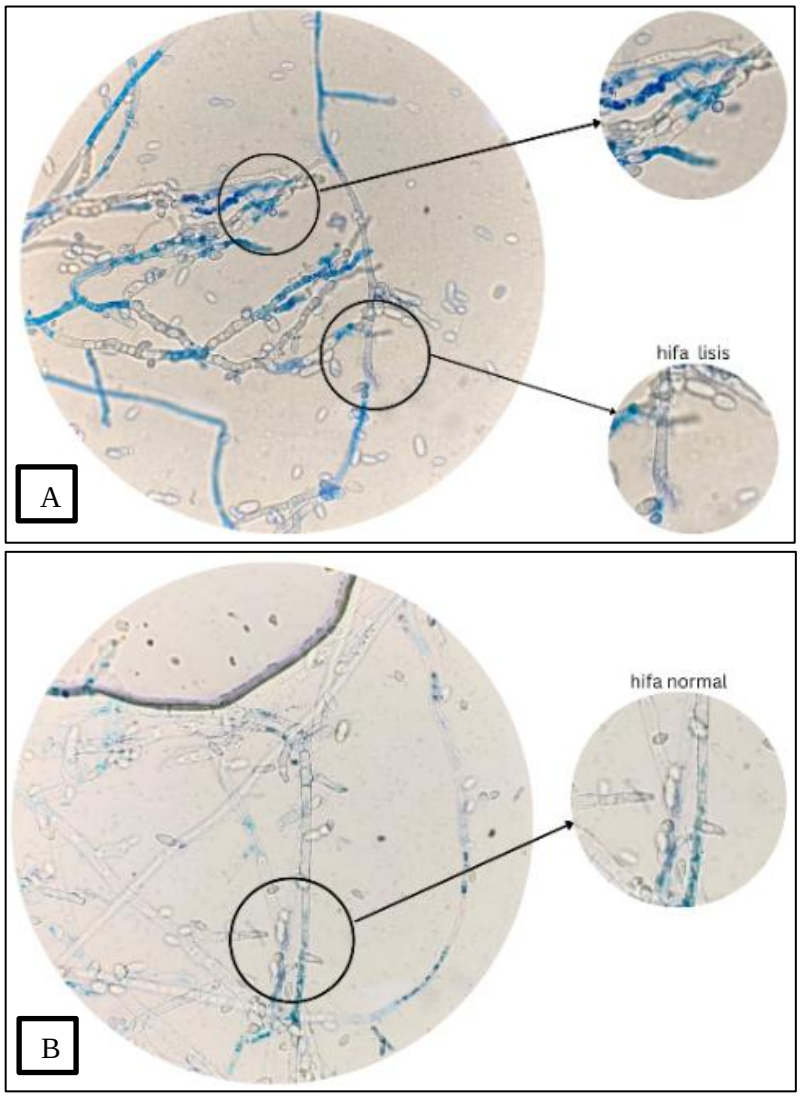

Figure 2. Observation of *Fusarium* sp. hyphae in an antagonism test (400x Magnification); Abnormal hyphae b) Normal hyphae

Incubation Period

Based on the results of the analysis of variance (ANOVA) and the 5% Honestly Significant Difference (HSD) test, the incubation period of *Fusarium* sp. showed a significant difference between treatment M3 and the other treatments. Meanwhile, no significant differences were observed among treatments M0, M1, M2,

and M4. The results presented in Figure 4.5 indicate that treatment M0 (control) had the shortest incubation period, lasting 8.52 days. The longest incubation period was observed in treatment M3, lasting 29.52 days, followed by treatment M2 at 12.96 days, treatment M4 at 10.68 days, and treatment M1 at 9.24 days. Treatment M3 was able to delay the incubation period in corn plants by up to 29.52 days, representing the greatest delay among all treatments. This effect may be attributed to the role of *Bacillus* sp. in protecting corn root tissues. The colonization of *Bacillus* sp. in plant roots may also stimulate the production of jasmonic acid and ethylene, which function to enhance plant resistance against pathogens. According to Djaenudin (2016), the average incubation period of *Fusarium* sp. in corn plants is approximately 10.67 days.

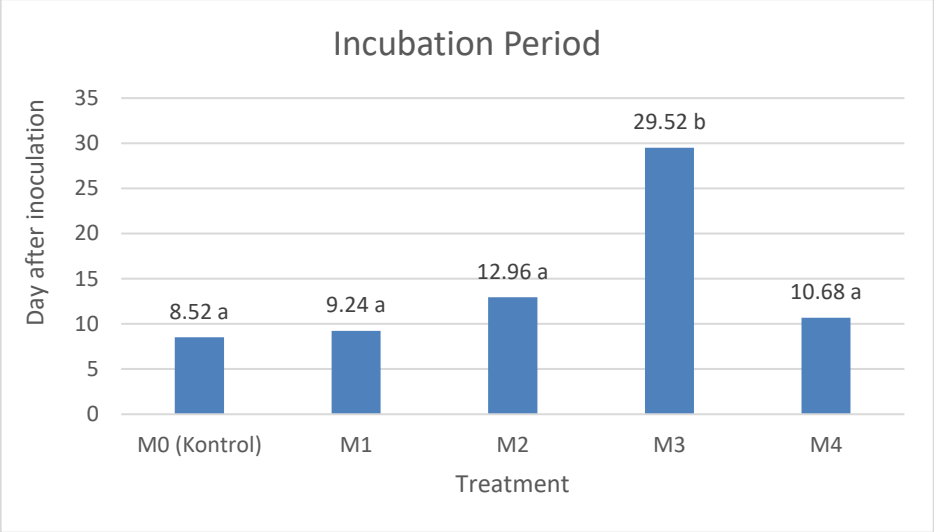

Figure 3. Incubation period of Fusarium Stem Rot in Maize Plants

Disease Intensity

Disease severity was assessed using a scoring method based on symptoms observed on maize leaves. The scoring data were subsequently analyzed using the disease severity formula to determine the percentage of disease severity in the maize plants. Based on the analysis of variance followed by the 5% Honestly Significant Difference (HSD) test, no significant differences among treatments were observed at 7 and 14 days after planting (DAP). However, significant differences began to emerge between 21 and 35 DAP. Treatment M3 exhibited the lowest disease severity, followed by treatments M2 and M1. In contrast, disease severity in treatment M4 was higher than that observed in treatments M1, M2, and M3. The control treatment (M0) consistently showed the highest disease severity across all observation time points.

Based on the results of the disease intensity analysis, no significant differences among treatments were observed at 7 and 14 days after sowing, likely due to the relatively long incubation period of *Fusarium* sp. The application of *Bacillus* sp. BTH-22 propagated in various alternative liquid media was able to suppress the intensity of Fusarium disease in maize plants. The results demonstrated that the application of *Bacillus* sp. BTH-22 propagated in different media had a significant effect on the intensity of *Fusarium* sp. infection throughout the observation period. The

colonization of *Bacillus* sp. BTH-22 in the root systems of maize plants, is suspected to be the primary factor influencing the intensity of *Fusarium* disease infection.

Table 4. Severity of *Fusarium* sp. Disease infection in maize plants

Treatment	Disease Intensity (%)				
	7	14	21	28	35
M0	0 a	0 a	0.36 c	0.60 c	0.75 c
M1	0 a	0 a	0.23 b	0.34 b	0.43 b
M2	0 a	0 a	0.14 ab	0.19 ab	0.35 ab
M3	0 a	0 a	0.05 a	0.09 a	0.12 a
M4	0 a	0 a	0.24 b	0.38 b	0.54 bc

Notes : Values followed by the same letter in the same column indicate no significant difference based on the HSD 5% test; M0: Control, M1: Nutrient broth medium, M2: Rice washing water medium, M3: Mung bean broth medium, M4: cowpea broth medium.

Bacillus sp. is also capable of inducing systemic resistance in plants through the mechanism of Induced Systemic Resistance (ISR). Activation of ISR enhances the expression of several plant defense enzymes, including peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, which are involved in the biosynthesis of lignin and phenolic compounds. Strengthening of plant cell walls through lignification can inhibit pathogen penetration and restrict its spread within plant tissues. ISR activation is further characterized by increased production of plant defense compounds, such as phenolics, tannins, and saponins, which contribute to cell wall reinforcement and suppress pathogen penetration and development. In addition, ISR enhances the activity of defense-related enzymes, including peroxidase and polyphenol oxidase, which play important roles in lignification and the synthesis of antimicrobial compounds (Choudhary et al., 2017).

CONCLUSION

The novelty of this study lies in the comparative evaluation of several inexpensive, locally available alternative liquid media for propagating *Bacillus* sp. BTH-22 while simultaneously assessing their influence on bacterial biocontrol performance against Fusarium stem rot in maize. The results demonstrate that bacterial population density does not necessarily correspond to the highest disease suppression, highlighting the importance of selecting propagation media based on both biomass production and biological efficacy. These findings provide new insights into optimizing economical mass-production systems for microbial biocontrol agents and support the development of sustainable disease management strategies for maize production.

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How To Cite This Article, with *APA style* :
Qafi, S. N., Purnawati, A., & Prasetyawati, E. T. (2026). Potential of *Bacillus* sp. (BTH-22) in Several Alternative Media for Controlling Fusarium Stalk Rot in Maize. *Jurnal Pembelajaran dan Biologi Nukleus*, *12*(2), 604-619. https://doi.org/10.36987/jpbn.v12i2.9342

Conflict of interest **:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions **:** All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Sabrina Nasrul Qafi**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.